Galectins expressed differently in genetically susceptible C57BL/6 and resistant BALB/c mice during acute ocular *Toxoplasma gondii* infection

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SUMMARY

Ocular toxoplasmosis (OT) caused by *Toxoplasma gondii* is a major cause of infectious uveitis, however little is known about its immunopathological mechanism. Susceptible C57BL/6 (B6) and resistant BALB/c mice were intravitreally infected with 500 tachyzoites of the RH strain of *T. gondii*. B6 mice showed more severe ocular pathology and higher parasite loads in the eyes. The levels of galectin (Gal)-9 and its receptors (Tim-3 and CD137), interferon (IFN)-γ, IL-6 and IL-10 were significantly higher in the eyes of B6 mice than those of BALB/c mice; however, the levels of IFN-α and -β were significantly decreased in the eyes and CLNs of B6 mice but significantly increased in BALB/c mice after infection. After blockage of galectin–receptor interactions by α-lactose, neither ocular immunopathology nor parasite loads were different from those of infected BALB/c mice without α-lactose treatment. Although the expressions of Gal-9/receptor were significantly increased in B6 mice and Gal-1 and -3 were upregulated in both strains of mice upon ocular *T. gondii* infection, blockage of galectins did not change the ocular pathogenesis of genetic resistant BALB/c mice. However, IFN-α and -β were differently expressed in B6 and BALB/c mice, suggesting that type I IFNs may play a protective role in experimental OT.

Key words: ocular toxoplasmosis, C57BL/6 mice, BALB/c mice, galectins, type I interferons, α-lactose.

INTRODUCTION

Ocular toxoplasmosis (OT), a potential blinding disease causes a high incidence of uveitis worldwide (Holland, 2003), which is caused by the obligate intracellular parasite *Toxoplasma gondii*. In spite of extensive research on epidemiology, immunology and pathophysiology of the disease (Maenz *et al.* 2014), many aspects of OT still remain unclear. After *T. gondii* infection, nearly all mouse lineages develop a T helper (Th)1-type immune response, although they present resistant or susceptible major histocompatibility complex (MHC) haplotypes (Gazzinelli *et al.* 1991, 1992). It has been demonstrated that C57BL/6 (B6) mice are more susceptible to the parasite than BALB/c mice (Suzuki *et al.* 1995).

Galectins, beta-galactoside-binding animal lectins, are differentially expressed by various immune cells as well as a wide range of other cell types (Liu and Rabinovich, 2010). So far, 15 members of the galectin family have been identified in vertebrates (Viguier *et al.* 2014). Some galectins such as galectin (Gal)-1, -3, -8 and -9 have wide tissue distribution, whereas others such as Gal-4, -5 and -6 exhibit tissue specificity (Panjwani, 2014). Galectins can regulate microbial invasion via acting as pathogen recognition receptors, and have multiple roles in both innate and adaptive immune responses (Vasta, 2009; Baum *et al.* 2014). Previous study has reported that Gal-3 can significantly alter the pathogenic course of *T. gondii* infection (Bernardes *et al.* 2006). T cell Ig and mucin domain-containing molecule-3 (Tim-3) was firstly identified as a molecule specifically expressed on interferon (IFN)-γ-producing $CD4^+$ Th1 and $CD8^+$ Tc1 (cytotoxic) cells in mice (Anderson *et al.* 2007). Studies further demonstrated that Tim-3 is expressed on cells of both innate and adaptive immune systems, including $CD4^+$ regulatory T cells, monocytes/macrophages, dendritic cells (DCs), and natural killer cells (Hu *et al.* 2016). Gal-9 is identified as a Tim-3 ligand and Gal-9/Tim-3 interaction acts as a specific inhibitor of Th1 and Th17 immune responses (Wu *et al.* 2014). Gal-9/Tim-3 interaction also plays a crucial role in immune regulation. Gal-9 administration significantly decreased viral load, inhibited mucus production and diminished lung immunopathology caused by respiratory syncytial virus infections (Lu *et al.* 2015). Studies demonstrated that Gal-9 also binds to CD44 (Chiba *et al.* 2012),

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CD137 (Liang and Qin, 2013) and protein disulphide isomerase (PDI) (Kojima *et al*. 2011). CD44 is a transmembrane adhesion molecule that is present on a wide variety of cell types, including leucocytes and parenchymal cells, and is an important player in leucocyte trafficking (Sunil *et al*. 2015). CD137 is a co-stimulatory member of the tumour necrosis factor (TNF) receptor family discovered on T cells undergoing activation (Kwon and Weissman, 1989).

So far, the immunopathological mechanisms that lead to ocular toxoplasmic infection remain largely unknown. Currently, it is not clear what role of galectins play in OT and whether manipulating galectin binding to their receptors can influence the magnitude and effectiveness of *T. gondii* immunity, their expression patterns in the context of OT have thus far not been elucidated. In addition, the pathway involved in the induction of IFN-I by the interaction of Gal-9 and its receptors in ocular immunopathogenesis during *T. gondii* infection have not been reported. In this study, we seek to compare the expression levels of Gal-1, -3, -8, -9, the receptors of Gal-9 (Tim-3, CD44, CD137 and PDI), IFN-I (IFN-α and -β), IFN-II (IFN-γ) and IL-10 in the eyes between *T. gondii*-susceptible B6 and -resistant BALB/c mice after ocular *T. gondii* infection, and the roles of galectins in BALB/c mice during ocular *T. gondii* infection were investigated with the blockage of galectins by α-lactose *in vivo*. We demonstrated that the signalling of Gal-9 and its receptors (Tim-3 and CD137) may involve in the development of OT in ocular *T. gondii*-infected B6 mouse model, and IFN-I production may play a protective role during ocular *T. gondii* infection.

MATERIALS AND METHODS

Mice, parasite, intravitreal infection and treatment with α-lactose

B6 and BALB/c mice, female, aged 5–6 weeks were obtained from the Experiment Animal Center of Sun Yat-sen University (Guangzhou, China). All animals were maintained in specific-pathogen-free environment and had free access to a commercial basal diet and tap water *ad libitum*. Tachyzoites of *T. gondii* RH strain stored in our laboratory were propagated by intraperitoneal passage in Kunming mice at 4–5-day intervals. For intravitreal infection, according to Charles *et al*. (2007) and modified, 1-μL parasite suspension containing 500 tachyzoits or the same volume of phosphate-buffered saline (PBS) was injected into one of the eye of per mice using a 10-μL Hamilton microsyringe. Some mice were injected intraperitoneally (i.p.) with 150 mM of α-lactose solution in PBS twice daily starting from 1 day post-infection (dpi) until the day mice were sacrificed. Animals were sacrificed at 12 h after the last treatment and their eyes and cervical lymph nodes (CLNs) were taken for further analysis (Sehrawat *et al*. 2010). A total of 30 mice of each strain were used in the experiments: eight mice were intravitreally injected with 500 *T. gondii* tachyzoites, eight mice were intravitreally injected with 500 *T. gondii* tachyzoites and treated with α-lactose; seven mice were injected i.p. with α-lactose alone; and seven mice were intravitreally injected with equal volume of PBS as negative controls. Mice infected with *T. gondii* were sacrificed by CO_2 asphyxiation for examination at 8 dpi. All experiments were performed in compliance with the requirements of the Animal Ethics Committee at Sun Yat-sen University.

Histopathological analysis

Mice were sacrificed by CO_2 asphyxiation at 8 dpi, and the eyes were harvested and immediately fixed in 10% buffered natural formaldehyde (Guangzhou Chemical Reagent Factory, China) for 48 h. Five-micrometre-thick sections of the organs from each mouse, stained with haematoxylin and eosin (H&E) (Sigma-Aldrich), were evaluated for histological changes and parasite proliferations.

Measurement of mRNA expression using quantitative real-time reverse transcription-polymerase chain reaction (qRT–PCR)

Total RNA was extracted from the eye and CLN samples of each mouse using RNA Extraction kit (TaKaRa Bio, Inc., Tokyo, Japan) according to the manufacturer's protocol. The quality of total RNA was analysed by running 5 μL of each RNA sample on a 1·0% agarose gel stained with ethidium bromide. The quantity of total RNA was estimated by measuring the ratio of absorbance at 260 and 280 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, DE, USA). First-strand cDNA was constructed from 1·0 μg of total RNA with oligo (dT) as primers using a PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa Bio, Inc.) following the manufacturer's protocol. cDNA was stored at −80 °C until use. To determine tissue mRNA levels of Gal-1, -3, -8 and -9, Tim-3, CD44, CD137, PDI, IFN-α, IFN-β, IFN-γ and IL-10, qRT–PCR was performed using SYBR Green QPCR Master Mix (TaKaRa Bio, Inc.) according to the manufacturer's instructions. For eye parasite loads, the levels of mRNA transcripts of *T. gondii* tachyzoite surface antigen 1 (SAG1) gene were measured by using qRT–PCR. Primers for the qRT–PCR are listed in Table 1. Briefly, a total of 10 μL reaction mixture contained 5·0 μL of SYBR® Premix Ex TaqTM (2×), 0·5 μL of each primer (10 pM), 3·0 μL of dH_2O and 1·0 μL of cDNA (0·2 $\mu g\,\mu L^{-1}$). Amplification was pre-denaturized for 30 s at 95 °C followed by 43 cycles of 5 s at 95 °C and 20 s at 60 °C with a LightCycler® 480 instrument (Roche

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Table 1. Primer sequences of mouse target cytokines and housekeeping genes used for quantitative real-time polymerase chain reaction (qRT–PCR) assays

Genes	Primer sequence (5′→3′)	Accession
SAG1	Forward primer ATGTCGCTTCTTAGCCGAGT	XM_002365028·1
	Reverse primer TCACAGGAAGTTGCTTCAGG	
IFN-α	Forward primer CTTTGGATTCCCGCAGGA	NM_010502·2
	Reverse primer TGTAGGACAGGGATGGCTTGA	
IFN-β	Forward primer TGAATGGAAAGATCAACCTCACCTA	NM_010510·1
	Reverse primer CTCTTCTGCATCTTCTCCGTCA	
IFN-γ	Forward primer GGAACTGGCAAAAGGATGGTGAC	NM_008337·4
	Reverse primer GCTGGACCTGTGGGTTGTTGAC	
IL-6	Forward primer CTGCAAGAGACTTCCATCCAG	NM_031168
	Reverse primer AGTGGTATAGACAGGTCTGTTGG	
IL-10	Forward primer AGCCGGGAAGACAATAACTG	NM_010548·2
	Reverse primer CATTTCCGATAAGGCTTGG	
Gal-1	Forward primer CGCCAGCAACCTGAATC	NM_008495·2
	Reverse primer GTCCCATCTTCCTTGGTGTTA	
Gal-3	Forward primer GCTACTGGCCCCTTTGGT	NM_001145953·1
	Reverse primer CCAGGCAAGGGCATATCGTA	
Gal-8	Forward primer GGGTGGTGGGTGGAACTG	NM_001199043·1
	Reverse primer GCCTTTGAGCCCCCAATATC	
Gal-9	Forward primer GTTGTCCGAAACACTCAGAT	NM_001159301·1
	Reverse primer ATATGATCCACACCGAGAAG	
Tim-3	Forward primer CCACGGAGAGAAATGGTTC	NM_134250·2
	Reverse primer CATCAGCCCATGTGGAAAT	
CD44	Forward primer TGCAGGTATGGGTTCATAGAAGG	NM_001039150·1
	Reverse primer GTGTTGGACGTGACGAGGA	
CD137	Forward primer CGTGCAGAACTCCTGTGATAAC	NM_001077508·1
	Reverse primer GTCCACCTATGCTGGAGAAGG	
PDI	Forward primer CGCCTCCGATGTGTTGGAA	NM_007952·2
	Reverse primer GAAGAACTCGACTAGCATGAGC	
β-actin	Forward primer TGGAATCCTGTGGCATCCATGAAAC	NM_007393·5
	Reverse primer TAAAACGCAGCTCAGTAACAGTCCG	

Diagnostics, AL, USA). Values are means from triplicate measurements, specific mRNA expression levels were normalized to that of the housekeeping gene, β-actin and the results are expressed as fold change compared with uninfected controls.

Statistical analysis

Results of experimental studies were reported as mean ± S.D. Independent-sample *t*-test and one way ANOVA followed by LSD *post hoc* test were performed to determine the statistical significance of differences between samples by using SPSS software for windows (version 19·0; SPSS, Inc., IL, USA). All graphs were performed using GraphPad Prism software and a value of $P < 0{\cdot}05$ was considered statistically significant.

RESULTS

B6 mice displayed significantly severer ocular pathology and higher ocular parasite loads than those in BALB/c mice

Compared with BALB/c mice, B6 mice developed severe ocular pathology characterized by an intense inflammatory response and severe necrosis as described previously, while BALB/c mice showed moderate inflammatory response and necrosis (Fig. 1A). To compare the parasite loads in the two strains of mice, the mRNA levels of *T. gondii* SAG1 were determined in the eyes. As expected, B6 mice showed a significantly higher parasite loads than those of BALB/c mice in the eyes and CLNs at 8 dpi ($P < 0{\cdot}001$) (Fig. 1B).

B6 mice displayed significantly higher mRNA expressions of Gal-1, -3, -8 and -9 and the receptors of Gal-9 (Tim-3 and CD137) in the eyes and CLNs than those in BALB/c mice

To determine the expression of galectins supposed to be involved in acute experimental OT, our results showed that compared with uninfected control mice, there were significantly increased mRNA expression levels of Gal-1 ($P < 0{\cdot}05$), -3 ($P < 0{\cdot}01$), -8 ($P < 0{\cdot}05$) and -9 ($P < 0{\cdot}01$) in the eyes of *T. gondii*-infected B6 mice at 8 dpi; significantly increased Gal-8 ($P < 0{\cdot}05$) in the CLNs of of B6 mice and significantly increased Gal-1 and -3 in the CLNs ($P < 0{\cdot}001$) of both B6 and BALB/c mice (Fig. 2A). As shown in Fig. 2B, Tim-3 levels were significantly increased in the eyes ($P < 0{\cdot}01$) and CLNs ($P < 0{\cdot}01$) of both B6 and BALB/c mice. CD137 levels were also significantly increased

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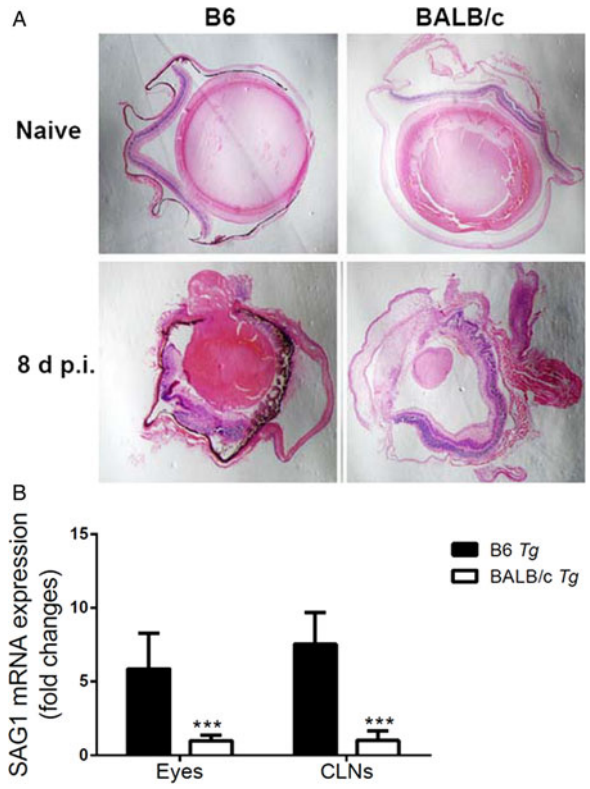

Fig. 1. Histological changes (A) and the mRNA expressions of SAG1 (B) in the eyes of *T. gondii*-infected B6 and BALB/c mice at 8 dpi. Naive mice, no histological alterations were observed; B6 mice-infected with *T. gondii* at 8 dpi showed severe damage in the eye; BALB/c mice showed moderate damage in the eye. Original magnification for eyes × 40, H&E stain. The mRNA expressions of SAG1 in the eye tissues of *T. gondii*-infected mice were measured by using qRT–PCR. Values are means from triplicate measurements, and data are presented as means ± S.D. The statistical analysis was performed by independent-sample *t*-test; ***$P < 0{\cdot}001$, *T. gondii*-infected B6 mice *vs* *T. gondii*-infected BALB/c mice. There were four mice per group, and data are representative of those from two experiments.

in the eyes and CLNs of both B6 ($P < 0{\cdot}001$) and BALB/c ($P < 0{\cdot}01$) mice. However, compared with BALB/c mice, there were significantly increased Gal-1 ($P < 0{\cdot}05$), -3 ($P < 0{\cdot}01$) and -9 ($P < 0{\cdot}01$) in the eyes and significantly increased Tim-3 ($P < 0{\cdot}05$) and CD137 ($P < 0{\cdot}001$ and $P < 0{\cdot}05$, respectively) in both the eyes and CLNs of B6 mice. These results indicate that ocular *T. gondii* infection leads to the induction of expressions of Gal-9 and its receptors (Tim-3 and CD137) in susceptible B6 mice, and the induction of Gal-1 or -3 in both susceptible B6 and resistant BALB/c mice.

BALB/c mice displayed significantly higher expressions of IFN-I (IFN-α and -β) in the eyes and CLNs than those in B6 mice

To assess the differences in cytokine production between the two strains of mice after ocular infection with *T. gondii*, the mRNA expressions of IFN-I (IFN-α and -β), IFN-II (IFN-γ), IL-10 and IL-6 in the eyes and CLNs were determined by using qRT–PCR. As shown in Fig. 3A, compared with uninfected control mice, there were significantly deceased IFN-α and -β in both the eyes ($P < 0{\cdot}05$) and CLNs ($P < 0{\cdot}05$) of B6 mice, but significantly increased IFN-α and -β in both the eyes ($P < 0{\cdot}05$) and CLNs ($P < 0{\cdot}05$) of BALB/c mice at 8 dpi; there were significant increased IFN-γ in the eyes ($P < 0{\cdot}001$) of both B6 and BALB/c mice, and in the CLNs ($P < 0{\cdot}001$) of B6 mice at 8 dpi. As shown in Fig. 3B, compared with uninfected control mice, there were significantly increased IL-6 and -10 in both the eyes ($P < 0{\cdot}001$) and CLNs ($P < 0{\cdot}01$) of B6 mice, and significantly increased IL-10 in the CLNs ($P < 0{\cdot}05$) of BALB/c mice at 8 dpi.

Ocular immunopathology in BALB/c mice was independent of galectins

To study the role of galectins in resistant BALB/c mice against ocular infection with *T. gondii*, BALB/c mice were intravitreally infected with *T. gondii*. As shown in Fig. 4, moderate inflammation and necrosis, and obvious tachyzoites or pseudocysts were observed in the eye tissues of BALB/c mice with or without α-lactose treatment. No significant differences of parasite loads in the eyes and CLNs were detected between the two groups.

Furthermore, the cytokine expressions were measured in BALB/c mice with or without α-lactose treatment. As shown in Fig. 5, compared with uninfected controls, the levels of IFN-α, -β and -γ in the eyes of *T. gondii*-infected BALB/c mice either with α-lactose treatment ($P < 0{\cdot}05$, $<0{\cdot}05$ and $<0{\cdot}001$, respectively) or without α-lactose treatment ($P < 0{\cdot}05$, $<0{\cdot}05$ and $<0{\cdot}001$, respectively) were significantly increased after ocular *T. gondii* infection; however, the expressions of IFN-α, -β and -γ had no significant differences between these two groups (Fig. 5A). Similarly, the levels of IL-6 and -10 in the eyes of *T. gondii*-infected BALB/c mice either with α-lactose treatment ($P < 0{\cdot}01$ and $<0{\cdot}05$, respectively) or without α-lactose treatment ($P < 0{\cdot}01$ and $<0{\cdot}05$, respectively) were significantly increased after ocular *T. gondii* infection but had no significant differences between these two groups (Fig. 5B). Because both ocular pathology and parasite loads showed no differences between *T. gondii*-infected BALB/c mice with or without α-lactose treatment, which suggests that galectins did not play an essential role for ocular immunopathology in genetic resistant BALB/c mice. In contrast, the severity of ocular pathology in susceptible B6 mice may be related to the expressions of galectins.

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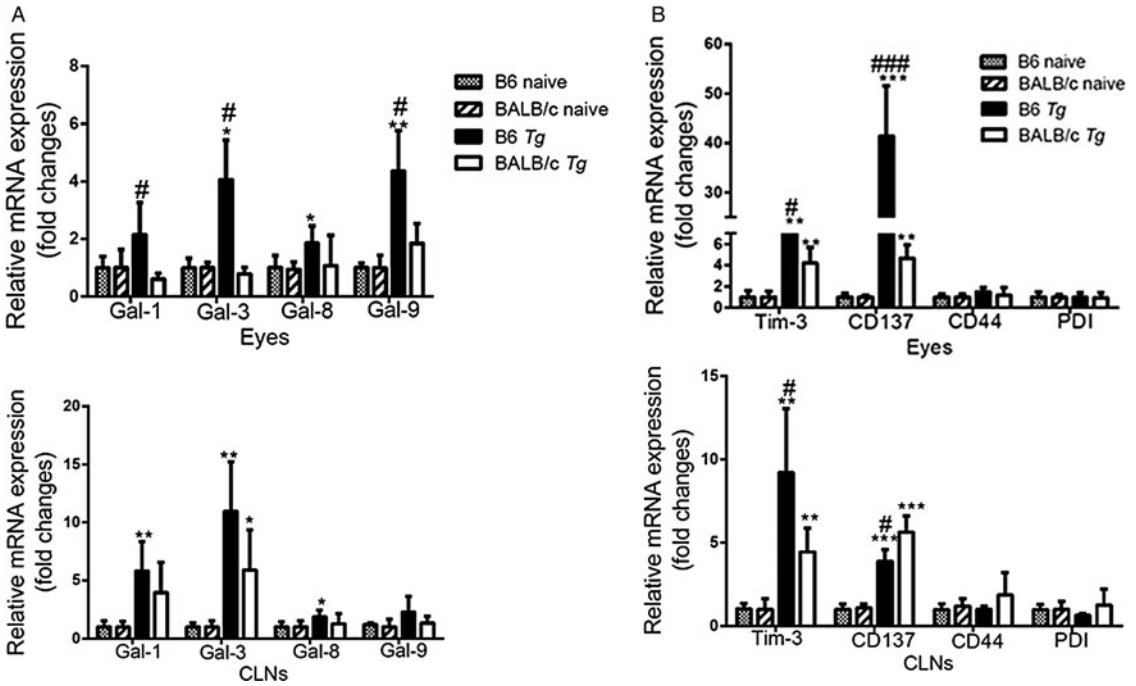

Fig. 2. The mRNA expressions of Gal-1, -3, -8 and -9 (A) and the receptors for Gal-9 (Tim-3, CD137, CD44 and PDI) (B) in the eyes and CLNs of *T. gondii*-infected mice at 8 dpi by using qRT–PCR. Values are means from triplicate measurements, and data are presented as means ± S.D. The statistical analysis was performed by independent-sample *t*-test; * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$ *vs* control group; # $P < 0{\cdot}05$, ### $P < 0{\cdot}001$, *T. gondii*-infected B6 mice *vs* *T. gondii*-infected BALB/c mice. There were four mice per group, and data are the representative of those from two experiments.

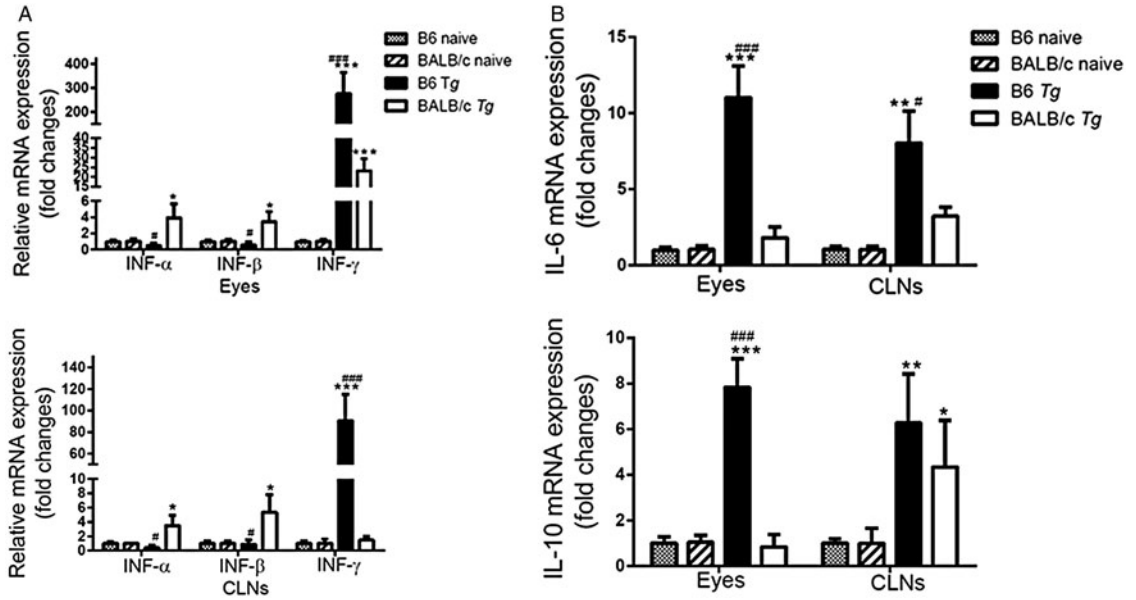

Fig. 3. The mRNA expressions of IFN-α, IFN-β, IFN-γ, IL-6 and IL-10 in the eyes and CLNs of *T. gondii*-infected mice at 8 dpi by using qRT–PCR. (A) IFN-α, IFN-β and IFN-γ; (B) IL-6 and IL-10. Values are means from triplicate measurements, and data are presented as means ± S.D. The statistical analysis was performed by independent-sample *t*-test; * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$ *vs* control group; # $P < 0{\cdot}05$, ### $P < 0{\cdot}001$, *T. gondii*-infected B6 mice *vs* *T. gondii*-infected BALB/c mice. There were four mice per group, and data are representative of those from two experiments.

DISCUSSION

Toxoplasma gondii infection is the most common cause of posterior uveitis worldwide (Holland, 2004). At present, laboratory mice continue to be the experimental model of choice for the investigation of most aspects of OT (Dukaczewska *et al.* 2015). Using eye inoculation by injecting 100 RH

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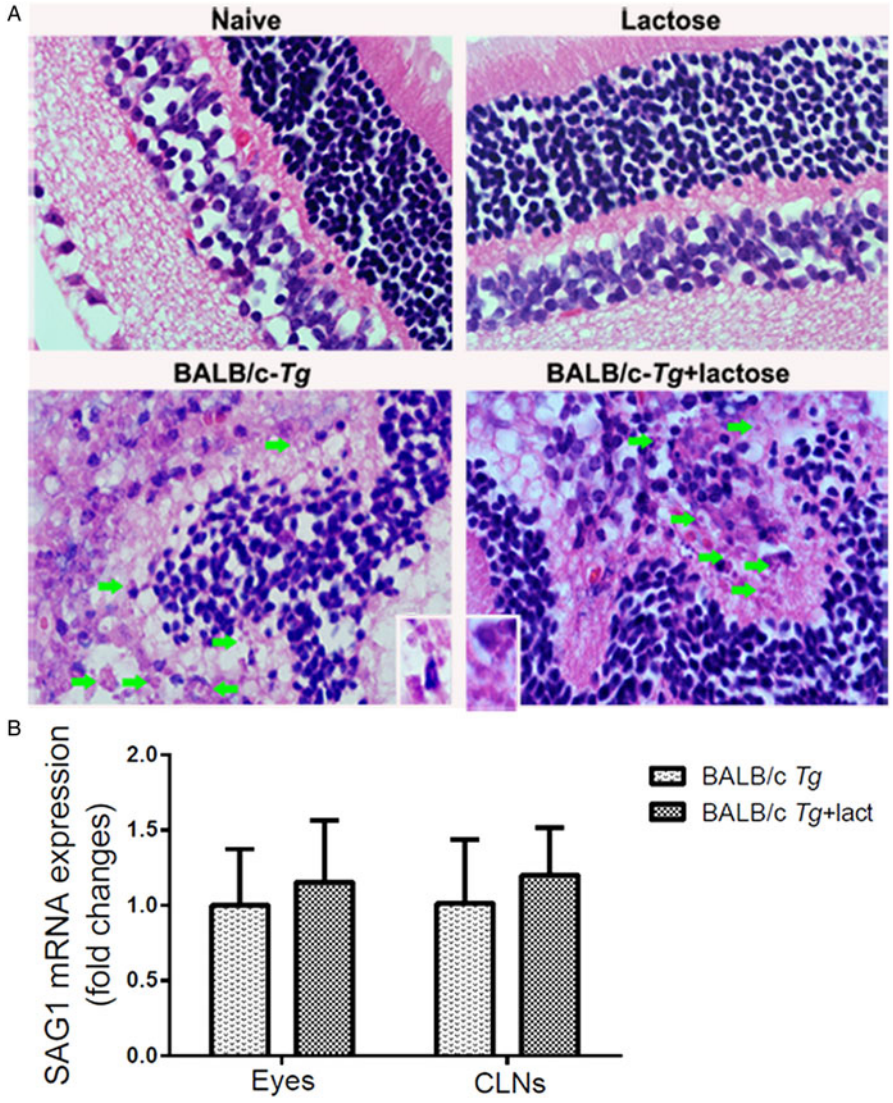

Fig. 4. Histological changes in the eyes (A) and SAG1 mRNA expressions in the eye and CLN tissues (B) of *T. gondii*-infected BALB/c mice with or without α-lactose treatment. Moderate histopathological changes were observed in the eyes of *T. gondii*-infected BALB/c mice with or without α-lactose treatment at 8 dpi. Tachyzoites or pseudocysts were indicated with arrows; their enlarged images were shown in small windows. Original magnification for eyes tissues × 1000. H&E stain. The SAG1 mRNA expressions in the eyes and CLNs were measured by using qRT–PCR. Values are means from triplicate measurements, and data are presented as means ± S.D. The statistical analysis was performed by independent-sample *t*-test. There were four mice per group, and data are representative of those from two experiments.

tachyzoites into the anterior chamber of eyes, our previous work demonstrated that genetic factors of the host as well as the parasite strain are critical in determining susceptibility to experimental OT in murine models (Lu *et al*. 2005). In this study, we investigated the role of galectins in the regulation of immune responses against ocular infection with *T. gondii* in susceptible B6 and resistant BALB/c mice by intravitreal injection of 500 RH tachyzoits. We firstly determined the expressions of Gal-1, -3, -8, -9 and its receptors (Tim-3, CD44, CD137 and DPI), IFN-α, IFN-β, IFN-γ, IL-6 and IL-10 in naive and *T. gondii*-infected B6 and BALB/c mice. Finally, blockage of galectins by treatment with α-lactose on the BALB/c background mice was used to investigate the role of galectins against ocular immunopathology. Taken together, our data indicate that acute ocular *T. gondii* infection results in increased Gal-1 and -3, especially Gal-9/Tim-3 and Gal-9/CD137 mRNA expressions in the eyes of susceptible B6 mice; however, the blockage of galectins did not change the severity of ocular toxoplasmic immunopathology and parasite loads in BALB/c mice. In addition, IFN-I may play a protective role in experimental OT but their expressions may be under genetic control.

In the current study, intraocular infection with tachyzoites of *T. gondii* induces elevated levels of IFN-γ that lead to the development of ocular immunopathology in both genetically susceptible B6 and resistant BALB/c mice; however, *T. gondii*-infected BALB/c mice developed lighter immunopathology.

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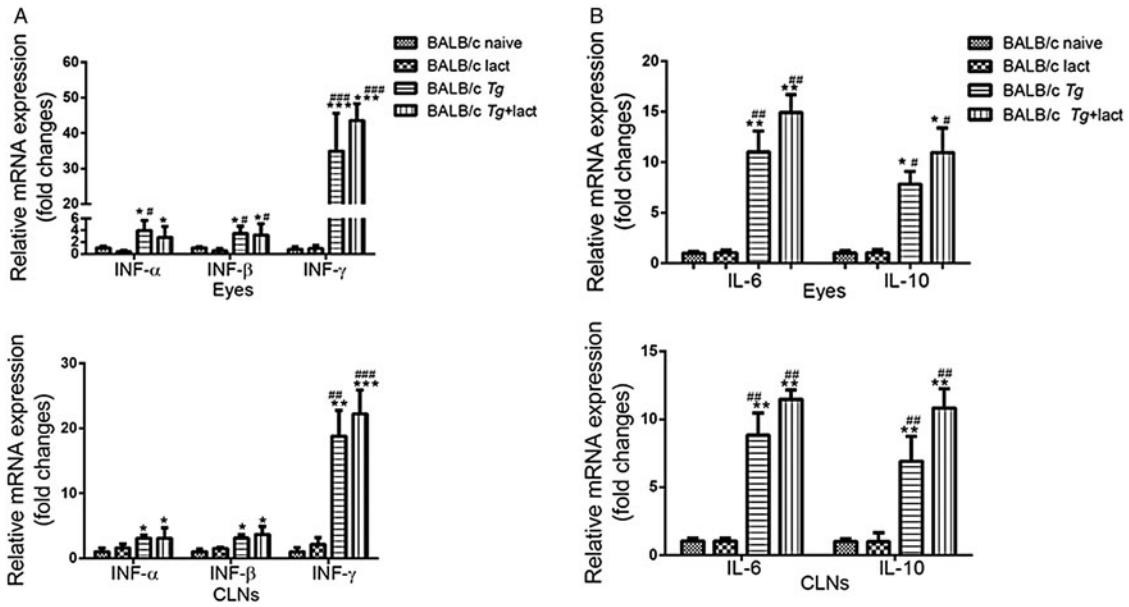

Fig. 5. The mRNA expressions of IFN-α, IFN-β, IFN-γ, IL-6 and IL-10 in the eyes and CLNs of *T. gondii*-infected BALB/c mice with or without α-lactose treatment at 8 dpi by using qRT–PCR. (A) IFN-α, IFN-β and IFN-γ; (B) IL-6 and IL-10. Values are means from triplicate measurements, and data are presented as means ± S.D. The statistical analysis was performed by one-way ANOVA with LSD's *post hoc* test; $*P < 0{\cdot}05$, $***P < 0{\cdot}001$ *vs* control group, $\#P < 0{\cdot}05$, $\#\#P < 0{\cdot}01$, $\#\#\#P < 0{\cdot}001$ *vs* α-lactose-control group. There were four mice per group, and data are representative of those from two experiments.

It has been reported that differentially expressed molecules in susceptible mice versus resistant mice are key to the development of immunopathology of *T. gondii* infection (Liesenfeld *et al*. 1996; Buzoni–Gatel *et al*. 2001; Liesenfeld, 2002). *T. gondii* infection elicits a strong Th1 response including with IFN-γ release (Suzuki *et al*. 1988). IFN-γ is required for an efficient activation of macrophages, and macrophages are of critical importance in antitoxoplasmic activity (Deckert-Schlüter *et al*. 1996). There is a remarkable difference in susceptibility to peroral infection with *T. gondii* among inbred strains of mice. IFN-γ mediates necrosis in the ilea of B6 mice after the ME49 strain of *T. gondii* infection, whereas the same cytokine plays a critical role in the resistance of genetically resistant BALB/c mice (Liesenfeld *et al*. 1996). Here we observed significantly higher levels of IFN-γ, IL-6 and IL-10 in the eyes and CLNs of B6 compared with BALB/c mice after ocular *T. gondii* infection. Following intraocular reinfection with the avirulent PRU strain of *T. gondii*, strongly enhanced production of IL-6 and IFN-γ in the aqueous humour of eyes was measured in B6 mice (Rochet *et al*. 2015). We previously reported that BALB/c mice when rendered deficient in IL-10 develop severe ocular immunopathology upon *T. gondii* infection via ocular inoculation (Lu *et al*. 2003), here we further demonstrated the key role of IL-10 in preventing the development of ocular immunopathology.

We found increased levels of Gal-1, -3, -8, -9, Tim-3 and CD137 in the eyes and Gal-8 in the CLNs of B6 mice; in addition, increased Gal-1 and -3, Tim-3 and CD137 were detected in the CLNs of both strains of mice after infection. After ocular *Pseudomonas aeruginosa* infection, subconjunctival injection of recombinant Gal-1 can significantly diminish corneal lesion severity through regulation of corneal infiltration of neutrophils and T cells, and modulation of Th17 and regulatory T (Treg) cell responses in the cornea as well as local draining lymph nodes (Suryawanshi *et al*. 2013). In corneal epithelium, an association of Gal-3 with cell surface mucins contributes to maintenance of the ocular surface epithelial barrier function (Argüeso *et al*. 2009). Gal-8 can promote the differentiation of Treg and Th2 cells by modulating IL-2 and TGFβ signalling (Sampson *et al*. 2016). Gal-8 attenuates the retinal pathology in a murine model of experimental autoimmune uveitis through enhancing the Treg cell response and inhibiting inflammation of the retina (Sampson *et al*. 2015). Tim-3/Gal-9 interaction plays a critical role at influencing the expression of herpes simplex virus induced ocular lesions (Sehrawat *et al*. 2009). Similarly, our data in this study indicated that Gal-9/Tim-3 and/or Gal-9/CD137 interaction may play a role in OT of B6 mice. However, after ocular *T. gondii* infection, no differences in ocular immunopathology or parasite loads were detected in BALB/c mice with α-lactose treatment compared to those without α-lactose treatment. Therefore, our data suggest that Gal-1, -3, -8 and -9 and its receptors were not key players in preventing ocular immunopathology induced by *T. gondii* in resistant BALB/c mice.

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We found significantly higher IFN-α/β expressions in *T. gondii*-infected BALB/c mice but significantly lower IFN-α/β expressions in infected B6 mice. IFN-I signalling through the IFN-α/β receptor (Ifnar) is critically important for innate immune defence (Sadler and Williams, 2008). A pivotal effect of Ifnar/IFN-β signalling in retinal microglia and macrophages that reduce chronic inflammation and pathological angiogenesis in age-related macular degeneration and thereby limit the development of choroidal neovascularization lesions (Lückoff *et al*. 2016). It has been reported that *T. gondii* is capable of eliciting IFN-α/β production in the serum of infected mice (Freshman *et al*. 1966). IFN-α/β production is an important factor associated with acute toxoplasmosis-induced immunosuppression in NMR1 mice (Swiss-type mice) infected with the RH strain of *T. gondii* (Diez *et al*. 1989). Using retinal pigment epithelial cell *in vitro* model system to evaluate *T. gondii* replication and the regulation of this replication by cytokines, it demonstrated that pretreatment of cultures with recombinant human IFN-α, -β or -γ prior to inoculation inhibited *T. gondii* replication in a dose-dependent manner, by the induction of indoleamine 2,3-dioxygenase (Nagineni *et al*. 1996). *In vivo* study showed that recombinant murine IFN-β protected mice against a lethal infection of *T. gondii* (Orellana *et al*. 1991). Inflammatory monocytes are major producers of IFN-β in mesenteric lymph nodes following oral infection of type II Prugniaud parasites in B6 mice, and mice lacking the receptor for IFN-1 (Toll-like receptors) show higher parasite loads and reduced survival (Han *et al*. 2014). Our data suggest that increased IFN-α/β levels in resistant BALB/c mice may facilitate the inhibition of *T. gondii* replication in this model.

In conclusion, the finding that B6 mice expressed higher levels of Gal-9 and its receptors (Tim-3 and CD137) than those in BALB/c mice after ocular *T. gondii* infection demonstrates that the expressions of Gal-9 and its receptors are genetically regulated in mice. However, blockage of galectin by α-lactose is not sufficient to change the ocular immunopathology in genetic resistant BALB/c mice. Deeper understanding of the role of galectins in *T. gondii* infection will shed new light on exploring the pathogenesis of OT. In addition, whether the markedly different expressions of IFN-α/β between intraocularly *T. gondii*-infected B6 and BALB/c mice are one of the mechanisms that they are genetically susceptible or resistant to the parasite needs to be further investigated.

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CONFLICT OF INTEREST

None.

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